

# Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry

Christian E. Zimmerman and Gordon H. Reeves

**Abstract:** Reproductive isolation between steelhead and resident rainbow trout (*Oncorhynchus mykiss*) was examined in the Deschutes River, Oregon, through surveys of spawning timing and location. Otolith microchemistry was used to determine the occurrence of steelhead and resident rainbow trout progeny in the adult populations of steelhead and resident rainbow trout in the Deschutes River and in the Babine River, British Columbia. In the 3 years studied, steelhead spawning occurred from mid March through May and resident rainbow trout spawning occurred from mid March through August. The timing of 50% spawning was 9–10 weeks earlier for steelhead than for resident rainbow trout. Spawning sites selected by steelhead were in deeper water and had larger substrate than those selected by resident rainbow trout. Maternal origin was identified by comparing Sr/Ca ratios in the primordia and freshwater growth regions of the otolith with a wavelength-dispersive electron microprobe. In the Deschutes River, only steelhead of steelhead maternal origin and resident rainbow trout of resident rainbow trout origin were observed. In the Babine River, steelhead of resident rainbow trout origin and resident rainbow trout of steelhead maternal origin were also observed. Based on these findings, we suggest that steelhead and resident rainbow trout in the Deschutes River may constitute reproductively isolated populations.

**Résumé :** L'examen de la phénologie de la fraye et la localisation des frayères chez les Truites arc-en-ciel (*Oncorhynchus mykiss*) anadromes et résidentes de la rivière Deschutes, en Oregon, ont servi à étudier l'isolement génétique entre les deux formes. La microchimie des otolithes a permis de reconnaître la présence des rejetons des truites anadromes et résidentes dans les populations adultes de la rivière Deschutes et dans la rivière Babine, en Colombie-Britannique. Pendant les 3 années de l'étude, la fraye des anadromes s'étendait de la mi-mars jusqu'à la fin de mai et celle des résidentes de la mi-mars à la fin d'août. Le date où 50% de la fraye des anadromes s'est terminée précédait de 9 à 10 semaines le moment équivalent chez les résidentes. Les anadromes choisissaient des sites de fraye plus profonds et à substrat plus grossier que les résidentes. L'origine maternelle des poissons a pu être déterminée par l'étude des rapports Sr/Ca dans les primordiums et les zones de croissance en eau douce des otolithes au moyen d'une microsonde électronique à dispersion de longueur d'onde. Dans la rivière Deschutes, seules des anadromes originaires de mères anadromes et des résidentes nées de mères résidentes ont été observées. Dans la rivière Babine, il y avait en plus des anadromes issues de mères résidentes et des résidentes provenant de mères anadromes. Nous concluons donc à partir de ces observations qu'il y a un isolement reproductif entre les formes anadrome et résidente des Truites arc-en-ciel dans la rivière Deschutes.

[Traduit par la Rédaction]

## Introduction

The term partial migration describes the phenomenon of populations divided into migratory and nonmigratory or resi-

dent individuals (Jonsson and Jonsson 1993). A variety of animal taxa from insects to birds and fish exhibit this phenomenon (Snyder and Dingle 1990; Berthold 1991; Wood 1995). Several species of salmonids exhibit partial migration. Within such populations, migratory behavior is diverse and well documented (Gross 1987; Thorpe 1987; Northcote 1992). Migrations exhibited by salmonids range from relatively short migrations between streams and lakes to long-distance migrations to the open ocean. Rainbow trout (*Oncorhynchus mykiss*; Neave 1944), coastal cutthroat trout (*Oncorhynchus clarki clarki*; Zimmerman et al. 1997), sockeye salmon (*Oncorhynchus nerka*; Wood 1995), Atlantic salmon (*Salmo salar*; Verspoor and Cole 1989), brown trout (*Salmo trutta*; Skaala and Nævdal 1989), and Arctic char (*Salvelinus alpinus*; Nordeng 1983) exhibit dual or multiple life-history forms that may reside in the same stream. Resident and migratory forms of a species may represent ecophenotypes within a

Received February 14, 2000. Accepted August 30, 2000.  
J15615

**C.E. Zimmerman.**<sup>1</sup> Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, U.S.A.

**G.H. Reeves.** U.S. Department of Agriculture Forest Service, Pacific Northwest Research Station, 3200 Southwest Jefferson Way, Corvallis, OR 97331, U.S.A.

<sup>1</sup>Author to whom all correspondence should be sent at the following address: c/o Forestry Sciences Laboratory, School of Aquatic and Fishery Sciences, University of Washington, 3200 Southwest Jefferson Way, Corvallis, OR 97331, U.S.A. e-mail: chris.zimmerman@orst.edu

single gene pool or they may represent reproductively isolated populations.

Foot et al. (1989) identified three possible genetic relationships between life-history forms. First, alternative life-history forms are genetically isolated and represent separate populations. Second, alternative life-history forms are not genetically distinct. Third, alternative life-history forms are genetically distinct within a local area but are more similar to one another than they are to their respective forms outside of the local area. Understanding the relation of sympatric life-history forms is critical to the study and management of such species.

Genetic divergence of populations requires reproductive isolation, which can result from barriers that isolate the populations or from the spatial or temporal separation of spawning activity. Leider et al. (1984) found evidence of reproductive isolation between the wild components of sympatric ecotypes (summer and winter runs) of steelhead (*O. mykiss*), the migratory form of rainbow trout. Although some gene flow between the populations occurred, they concluded that temporal segregation of spawning had led to reproductive isolating mechanisms that were sufficient to maintain the racial identity of the populations. In a study of brown trout, Baglinière et al. (1989) found spatial separation of spawning between resident and migratory populations in a small stream in France. This segregation of spawning habitat had not led to reproductive isolation, as the progeny of resident and migratory spawners could contribute to either life-history form. Identifying the extent to which migratory forms contribute to the spawning population of resident forms and vice versa is required to determine reproductive isolation.

Analysis of otolith microchemistry provides a means of determining whether adult spawners are the progeny of migratory or nonmigratory forms. If migratory and nonmigratory trout represent ecophenotypes within a single gene pool, migratory adults of resident maternal origin and resident trout of migratory maternal origin would be commonly encountered. If the two life-history forms are reproductively isolated, migratory trout of resident trout maternal origin and residents of migratory trout maternal origin would not be present. Otolith microchemistry can be used to identify maternal origin based on examination of the ratio of strontium (Sr) and calcium (Ca). Sr, an element with binding characteristics similar to Ca, is substituted for Ca in the calcium carbonate matrix of the otolith at levels relative to the concentration in the environment (Kalish 1990). The concentration of Sr is greater in seawater than in fresh water. Therefore, analysis of Sr/Ca ratios across the otolith of a fish can describe the migrational history of that fish. Radtke (1995) and Babaluk et al. (1997) used Sr/Ca ratios to describe the migration history of Arctic char. Secor et al. (1995) confirmed that changes in the Sr/Ca ratios in otoliths of striped bass (*Morone saxatilis*) corresponded to changes in ambient-water chemistry. Further, comparison of Sr/Ca ratios in the primordia and freshwater growth region can be used to determine maternal origin (resident or anadromous), based on the assumption that primordia composition reflects the environment in which yolk precursors develop (in the ocean for anadromous forms) (Kalish 1990). Using these techniques, Rieman et al. (1994) were able to determine the maternal or-

igin of juvenile sockeye salmon and their resident form, kokanee, in the Snake River, Idaho.

Rainbow trout are native to western North America, from northwest Mexico to the Kuskokwim River, Alaska (Scott and Crossman 1973). Resident and migratory life histories have been described throughout this range. The anadromous form, or steelhead, spawns in streams or rivers with access to the ocean. Juvenile steelhead rear in freshwater habitats for 1–3 years before migrating to the ocean. After 1–3 years, the adult steelhead returns to fresh water to spawn. Resident rainbow trout remain in fresh water throughout the life cycle. The relationship of migratory and resident forms of rainbow trout has long confused biologists. Variation in appearance, morphology, and behavior—including migratory differences—led to their original classification as a different species (Jordan and Evermann 1905; Behnke 1992).

In this study, we compared the spatial and temporal distribution of spawning by sympatric steelhead and resident rainbow trout in the Deschutes River, Oregon. Understanding the temporal and spatial use of spawning habitat provides an indication of potential reproductive isolation between the two life-history forms. Next, we compared the maternal origin of adult steelhead and resident rainbow trout from the Deschutes River and the Babine River, British Columbia. If steelhead and rainbow trout represent ecophenotypes within a single gene pool, steelhead of resident maternal origin and resident rainbow trout of steelhead maternal origin would be commonly encountered. If the two life-history forms are reproductively isolated, steelhead of rainbow trout maternal origin and rainbow trout of steelhead maternal origin would not be present.

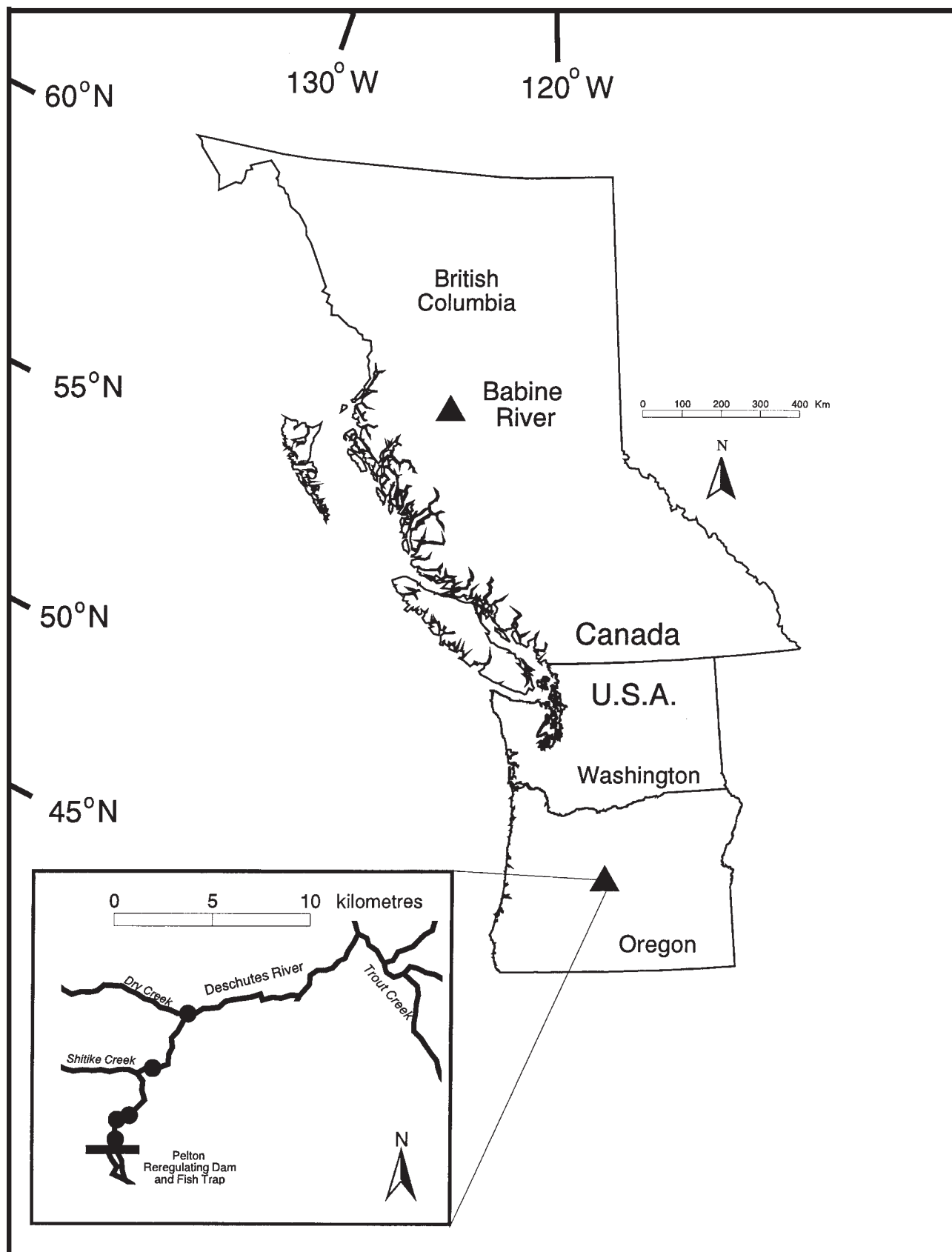
## Materials and methods

### Study site

The Deschutes River (44°40'N, 120°57'W) is a tributary of the Columbia River and drains 26 700 km<sup>2</sup> of north-central Oregon (Fig. 1). Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead once inhabited much of the basin but, since 1968, have been limited by the Pelton – Round Butte Dam complex to the lower 160 km. The lower Deschutes River supports populations of spring and fall chinook salmon, steelhead, rainbow trout, mountain whitefish (*Prosopium williamsoni*), bull trout (*Salvelinus confluentus*), northern pikeminnow (*Ptychocheilus oregonensis*), suckers (*Catostomus* spp.), speckled dace (*Rhinichthys osculus*), and sculpins (*Cottus* spp.).

The spawning-survey portion of this study focused on the 21 km of mainstem river between the Pelton Reregulating Dam at river kilometre (RK) 160 and the Trout Creek campground at RK 139 (Fig. 1). Through this reach, the river averages 67 m in width (Huntington 1985). The Deschutes River is well known for its stability of flow, which is more uniform than any other river of its size (Henshaw et al. 1914). Flows in the study area are controlled by dams and ranged from 115 to 179 m<sup>3</sup>·s<sup>-1</sup>. During 1995, the entire 21 km of river was examined. The study area was subsequently limited to five study sites, because a large number of rainbow trout redds were encountered. Five island-associated side channels ranging in length from 45 to 225 m and in width from 5 to 20 m were chosen in which to directly examine spawning. The five selected sites contained 68% of all steelhead redds constructed during 1995. We concluded that they adequately represented the spawning habitats of the two fishes. Helicopter surveys in 1995 and 1996 did not indicate that any significant aggregations of steelhead spawning oc-

**Fig. 1.** Map showing otolith-sampling locations (solid triangles) and the spawning-survey study area, Deschutes River, Oregon. Spawning-survey study sites are indicated by solid circles.



curred outside the five study sites. Only one additional steelhead redd was observed during the 1995 helicopter survey that had been missed in the stream surveys.

### Spawning surveys

Spawning surveys were made weekly in 1995, 1996, and 1997. Surveys began in the second week in March and continued until the cessation of spawning, which was generally the second week of August. We made periodic examinations of the study reach prior to the start of data collection, to determine if spawning began earlier, but none was observed prior to the second week of March.

The observer walked upstream through the study site and mapped the location of all new redds. The origin of redds (steelhead or rainbow trout) was determined on the basis of sightings of fish in the vicinity of the redd or the size of the redd. Steelhead and resident rainbow trout observed adjacent to redds or actively spawning were identified based on coloration, body shape, and size. Steelhead were larger, more fusiform, and had less spotting than resident rainbow trout. The average length of adult steelhead in the Deschutes River ranges from 61 to 69 cm (Olsen et al. 1994). Adult steelhead range in length from 48 to 80 cm, with more than 90% of the population being longer than 54 cm. Adult rainbow trout range in length from 16 to 50 cm, with over 70% of the population ranging between 20 and 35 cm (Schroeder and Smith 1989). On each spawning survey, new redds and locations of fish were recorded on maps of each site. New redds were identified by the presence of actively spawning fish or by coloration of the substrate and algal growth. Gravel was cleaner and brighter in new redds than in older redds or where there were no redds. Within 1 week, growth of periphyton and settlement of fine sediment would change the color of the redd. By observing known redds on a daily and weekly basis, a practiced observer could gauge the age of unknown redds based on these characteristics. To examine temporal distribution of spawning, the percent of total redds constructed by each life-history form was plotted against week. A Kolmogorov-Smirnov two-sample test was used to determine the significance of differences in the timing of steelhead and rainbow trout spawning by comparing the cumulative frequency distributions within each year.

### Microhabitat measurements of redd sites

To describe microhabitat features of the location of redds used by spawning steelhead and resident rainbow trout, we measured water depth and water velocity over redd, and estimated gravel size in the tailspill of redds. For this portion of the study, a redd was identified as either a steelhead or resident rainbow trout redd based on the presence of actively spawning fish or single females holding in the water column over the redd, and only redds that could be positively identified to life-history type were included. All positively identified steelhead redds were measured, and a stratified sample of positively identified resident rainbow trout redds were measured. A total of 28 steelhead redds and 52 resident rainbow trout redds were examined over the 3 years of the study. We measured water depth and velocity at the upstream edge of the redd pit. Water depth was measured to the nearest 1 cm with a wading staff. We measured water velocity at 60% of water depth to the nearest  $1 \text{ cm}\cdot\text{s}^{-1}$ , using an electromagnetic current meter. The size of the gravel in the tailspill was estimated by measuring the predominant size of material in the surface layer of the tailspill. To confirm that redd size could appropriately be used for identifying unknown redds, the length of each positively identified redd was measured from the upstream edge of the pit to the most downstream edge of the tailspill and the width of the redd was measured at the widest cross section perpendicular to the flow.

One-way analysis of variance was used to determine the significance of differences in mean characteristics of steelhead and rainbow trout redds. Stepwise discriminant function analysis was performed

to describe the association of water depth, water velocity, and substrate size in the selection of redd sites by the two life-history forms.

### Otolith collection

Sagittal otoliths were collected from adult rainbow trout and steelhead in the Deschutes River, Oregon, and the Babine River weir area, British Columbia (Fig. 1). The Babine River ( $54^{\circ}40'N$ ,  $126^{\circ}00'W$ ), a tributary of the Skeena River, is located in north-central British Columbia. The Babine River Counting Fence is located 360 km from the mouth of the Skeena River just downstream of Nilkitkwa Lake. Otoliths were collected in the Deschutes River from wild adult steelhead ( $n = 20$ ) returning to the Pelton Fish Trap at RK 160 and from carcasses encountered during spawning surveys. Adult rainbow trout ( $n = 38$ ) were collected from a 3-km segment of the riverbank with a drift-boat-mounted electroshocker and from carcasses encountered during spawning surveys. Only fish older than age 2 were included in these analyses. Rainbow trout in the Deschutes River reach maturity at age 3 and steelhead smolt at age 1 or 2 (Olsen et al. 1994; Schroeder and Smith 1989). By including only mature post-smolt ages we were able to ensure that we were comparing adult steelhead and resident rainbow trout. Babine River steelhead otoliths ( $n = 24$ ) were collected from mortalities encountered at the Babine Lake salmon-counting weir; resident rainbow trout ( $n = 9$ ) were collected from the stream in the vicinity of the weir. All resident rainbow trout from the Babine River were age 4 or 5 and ranged in length from 350 to 495 mm. Although most steelhead in the Babine River smolt at age 3, a small proportion smolt at ages 4 and 5 (Narver 1969). The back calculated lengths-at-age of steelhead at ages 4 and 5 ranged from 179 to 246 mm (Narver 1969).

### Otolith preparation and microchemical analysis

One sagittal otolith from each fish was mounted sulcus side down with Crystal Bond 509 on a microscope cover slip attached to a standard microscope slide. The otolith was then ground with 1200-grit sandpaper in the sagittal plane to the level of the nucleus. The mounting medium was heated and the otolith turned sulcus side up. The otolith was then ground with 1200-grit and 2000-grit sandpaper in the sagittal plane to the level of the primordia and polished with a slurry of  $0.05\text{-}\mu\text{m}$  alumina paste. The cover slip was then cut with a scribe, so that several prepared otoliths could be mounted on a petrographic slide for microprobe analysis. The slide containing several otoliths was rinsed with deionized water, air-dried, and coated with a  $400\text{-}\text{\AA}$  carbon layer ( $1 \text{ \AA} = 0.1 \text{ nm}$ ).

Elemental analysis was conducted with a Cameca SX-50 wavelength dispersive microprobe. A 15-kV, 50-nA,  $7 \text{ }\mu\text{m}$  diameter beam was used for all analyses. Strontianite ( $\text{SrCO}_3$  (USNM R10065)) and calcite ( $\text{CaCO}_3$  (USNM 136321)) were used as standards for Sr and Ca, respectively. The two elements were analyzed simultaneously, and a counting time of 40 s was used to maximize precision (Toole and Nielsen 1992). Sr was measured using the TAP crystal and Ca was measured using the PET crystal.

Otolith regions were classified as primordia, freshwater growth region, and saltwater growth region, based on growth rates inferred from banding patterns. The freshwater growth region included the area between the nucleus and the point of saltwater entry for anadromous fish or the area between the nucleus and the last annulus for resident fish. The saltwater growth region included the area following the freshwater growth region and preceding the last annulus for anadromous fish. Identification of freshwater and saltwater annuli was based on the methods of McKern et al. (1974). On all otoliths, microprobe sample points included all primordia (4–12 per fish) and transects of at least 10 points in both the freshwater and saltwater (in steelhead) growth regions. In most samples, the freshwater growth region was sampled within the summer growth of the



**Table 1.** Number of new redds identified by week in five study sites, Deschutes River, Oregon.

Month	Week-of-the-year	1995		1996		1997	
		Steelhead redds	Rainbow trout redds	Steelhead redds	Rainbow trout redds	Steelhead redds	Rainbow trout redds
March	11	0	0	0	0	0	0
	12	8	0	0	0	3	0
	13	6	18	1	2	7	0
April	14	10	17	1	2	*	*
	15	8	8	2	7	24	0
	16	9	14	4	18	10	14
	17	5	18	4	10	9	17
	18	0	23	1	28	*	*
May	19	2	79	5	37	3	34
	20		129	0	37	2	57
	21		126	3	73		68
	22		118		85		79
	23		152		138		106
June	24		231		218		157
	25		219		173		138
	26		86		203		171
	27		94		190		170
July	28		43		114		117
	29		29		90		71
	30		15		48		26
	31		8		18		11
August	32		3		13		5
	33		0		0		0
Total		48	1430	21	1504	58	1241

\*No survey, owing to high flows.

first year. On a subsample of otoliths, a transect of sample points bisecting a primordium and continuing to the edge of the otolith (life-history transect) was conducted for comparison with expected transects of Sr/Ca ratios described by Kalish (1990). Maternal origin was determined by comparing Sr/Ca ratios in the primordia with Sr/Ca ratios in the freshwater growth region. A fish was determined to be of anadromous maternal origin, if the Sr/Ca ratio in the primordia was significantly higher than that in the freshwater growth region, based on an unpaired one-tailed  $t$  test with  $\alpha = 0.05$ .

## Results

### Timing and duration of spawning

The total numbers of steelhead redds observed ranged from 21 to 58 in the five study sites (Table 1). The total numbers of rainbow trout redds ranged from 1241 to 1504 (Table 1). Steelhead and resident rainbow trout spawning began about the same time but the spawning period was shorter for steelhead than for resident rainbow trout. Steelhead spawning occurred between the middle of March and the end of May, and trout spawning occurred between the end of March and the end of August. Only 9–15% of the total resident rainbow trout redds were observed during the period of steelhead spawning. The time of 50% spawning by steelhead occurred during week-of-the-year 14, 16, and 14 in 1995, 1996, and 1997, respectively, (Fig. 2). The time of 50% spawning by resident rainbow trout occurred during week-of-the-year 23 in 1995 and 24 in 1996 and 1997 (Fig. 2).

The distribution in time of steelhead and rainbow trout spawning was significantly different in all 3 years (Kolmogorov–Smirnov two-sample test,  $P < 0.0001$ ).

### Microhabitat measurements

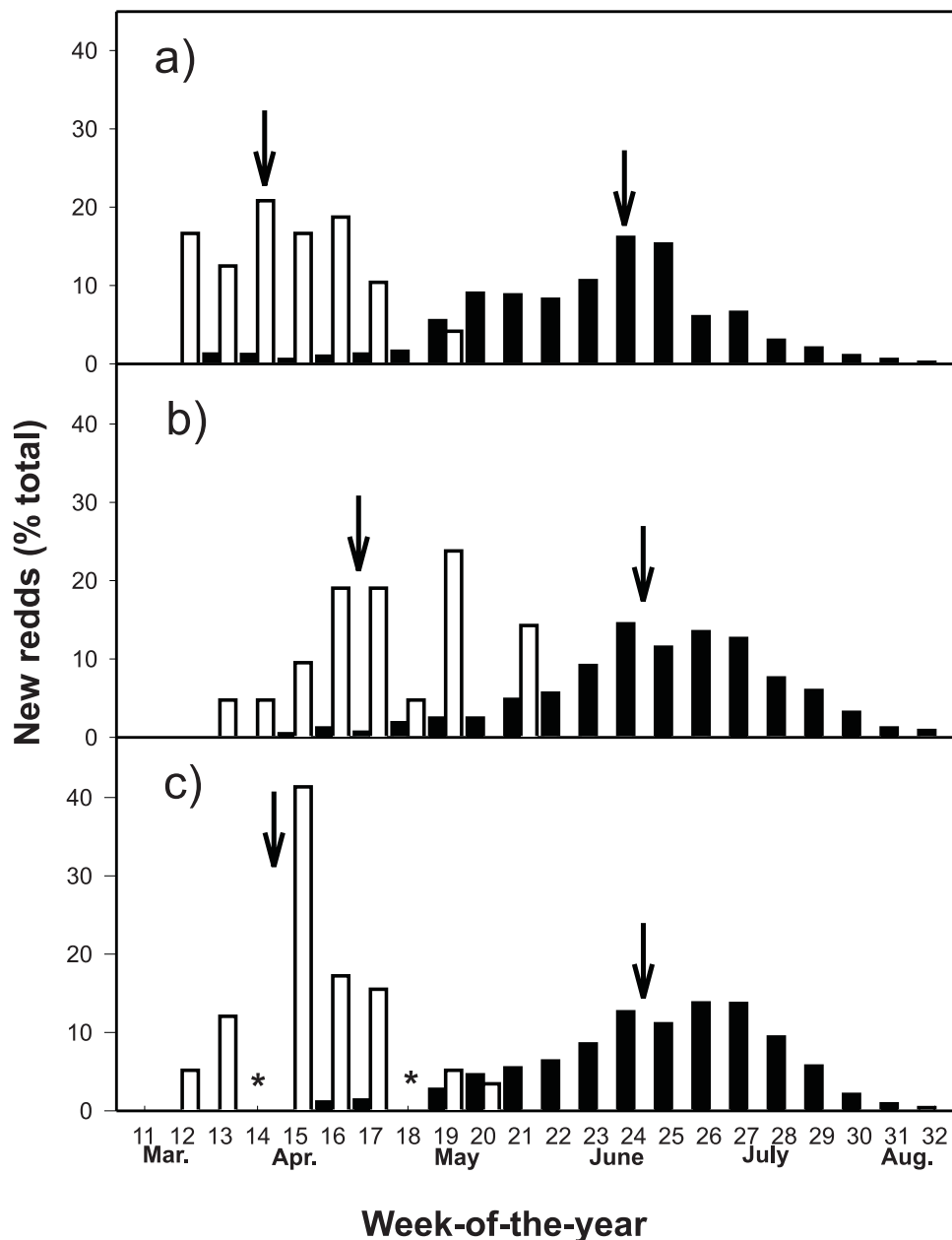
Steelhead and rainbow trout spawned in areas with different features. Steelhead redds were significantly larger than rainbow trout redds ( $P < 0.001$ ). Steelhead redds were in deeper water ( $P < 0.001$ ) and had larger substrate ( $P < 0.001$ ) than rainbow trout redds (Table 2). However, there was no difference ( $P > 0.05$ ) in the water velocity over steelhead and rainbow trout redds (Table 2).

Stepwise discriminant function analysis indicated that water depth and substrate size contributed significantly to separation of steelhead and resident rainbow trout. One-way analysis of variance indicated significant differences between steelhead and resident rainbow trout for the first discriminant function ( $F_{[1,78]} = 22.866$ ,  $P < 0.0001$ ). The first discriminant function accounted for 100% of the variation. Wilks'  $\lambda$ , a measure of the function's discriminating power, was 0.7733 ( $P < 0.001$ ). Water depth and gravel size in the tailspill were positively correlated ( $P < 0.01$ ) with the discriminant scores. Reclassification of redds by the discriminant function correctly identified 64% of steelhead redds and 72% of resident rainbow trout redds.

### Otolith microchemistry and population structure

Life-history transects of steelhead were similar to those described by Kalish (1990) for anadromous salmonids, with

**Fig. 2.** Percent of total redds observed by week-of-the-year, Deschutes River, Oregon, in 1995 (a), 1996 (b), and 1997 (c). Open bars are steelhead redds and solid bars are resident rainbow trout redds. Arrows indicate timing of 50% spawning and asterisks indicate lack of data due to high flows.



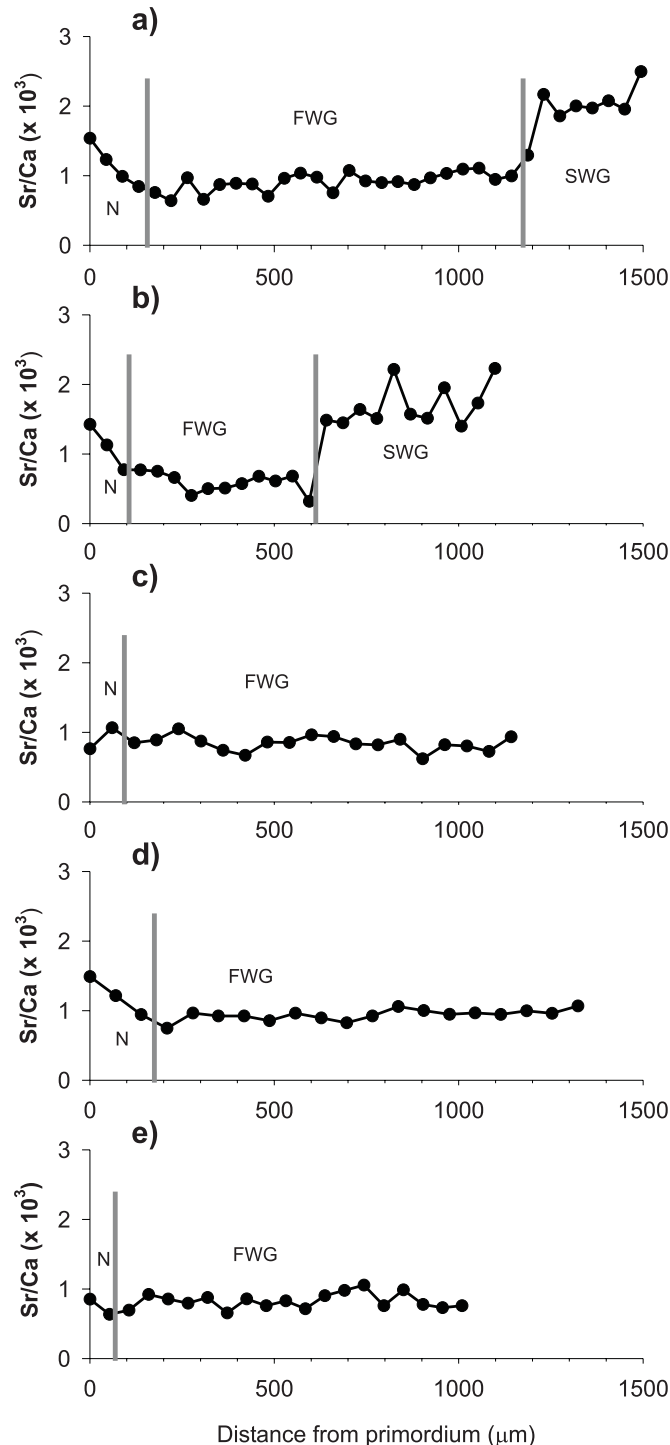
**Table 2.** Characteristics (mean  $\pm$  SE) and one-way analysis of variance of 28 steelhead and 52 rainbow trout redds, Deschutes River, Oregon.

Variable	Steelhead	Rainbow trout	F ratio	P
Water depth (cm) adjacent to pit	54.07 $\pm$ 2.74	42.58 $\pm$ 1.89	3.51	0.0007
Mean water velocity (cm·s <sup>-1</sup> ) adjacent to pit	71.43 $\pm$ 3.41	63.35 $\pm$ 2.51	1.43	ns
Gravel size (mm) in tailspill	32.50 $\pm$ 1.98	25.10 $\pm$ 1.11	3.53	0.0007
Redd length (m)	2.08 $\pm$ 0.14	1.50 $\pm$ 0.05	4.77	0.00001
Redd width (m)	1.18 $\pm$ 0.11	0.83 $\pm$ 0.03	3.81	0.0003

lower Sr/Ca ratios in the freshwater growth regions and increased Sr/Ca ratios in saltwater growth regions (Figs. 3a and 3b). Life-history transects of resident rainbow trout were

characterized by constant low Sr/Ca ratios (Figs. 3c and 3e). Adult resident rainbow trout of steelhead maternal origin were characterized by increased Sr/Ca ratios in the primordia

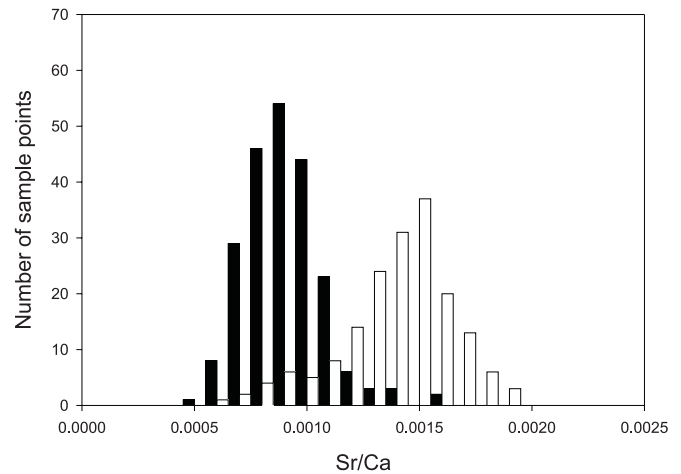
**Fig. 3.** Transects of otolith Sr/Ca ratios measured from a single primordium to the otolith edge. Each point represents a single measurement. (a) Deschutes River adult steelhead. (b) Babine River adult steelhead. (c) Deschutes River adult rainbow trout. (d) Babine River adult rainbow trout whose maternal parent was a steelhead. (e) Babine River adult rainbow trout whose maternal parent was a resident rainbow trout. N, nucleus; FWG, freshwater growth region; SWG, saltwater growth region.



and nucleus and constant lower Sr/Ca ratios throughout the rest of the otolith (Fig. 3d).

The relation between the life history of an individual and

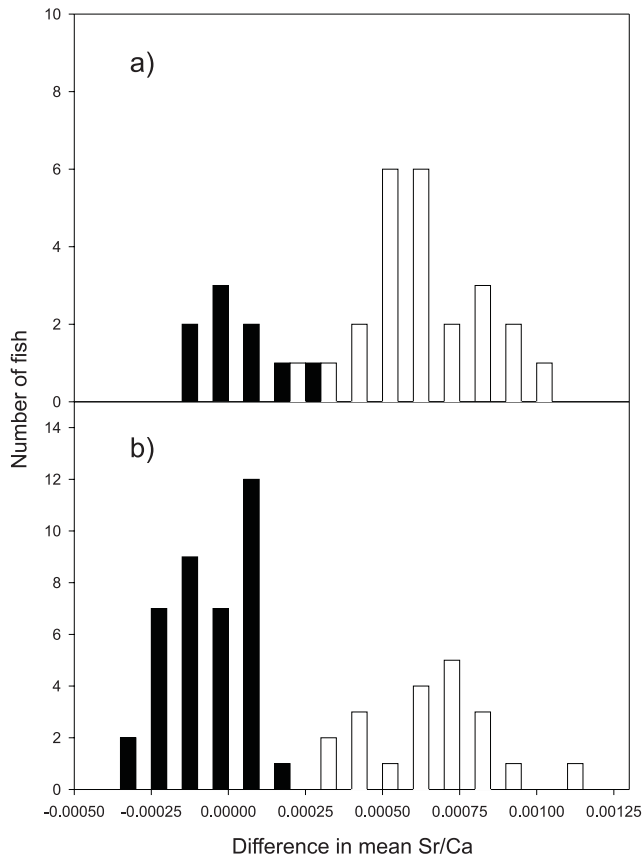
**Fig. 4.** Frequency distribution of Sr/Ca ratios in primordia of resident rainbow trout (solid bars) and steelhead (open bars) from the Deschutes River, Oregon.



the life history of the maternal parent differed between the Deschutes and Babine rivers. Of the 20 steelhead otoliths examined from the Deschutes River, all had significantly higher Sr/Ca ratios in the primordia than in the freshwater growth region, indicating that at least the maternal parent of each fish was a steelhead (all  $P < 0.0001$ ). None of the 38 resident rainbow trout otoliths from the Deschutes River had significantly higher Sr/Ca ratios in the primordia than in the freshwater growth region (all  $P > 0.05$ , range 0.16–0.49), indicating that at least the maternal parent of each fish was a resident rainbow trout. The relation between individual life history and the life history of the maternal parent of fish in the Babine River was less consistent than in the Deschutes River. Of the 24 steelhead otoliths from the Babine River weir area examined, all but one had significantly higher Sr/Ca ratios in the primordia than in the freshwater growth region ( $P < 0.05$ , range 0.001–0.02), indicating steelhead maternal origin. The Sr/Ca ratio in the primordia of one Babine River steelhead was not significantly higher than that in the freshwater growth region ( $t = 1.46$ ,  $P = 0.09$ ), indicating that the maternal parent was a resident rainbow trout. Two of the nine resident rainbow trout otoliths from the Babine River weir area had significantly higher Sr/Ca ratios in the primordia than in the freshwater growth region ( $P < 0.001$ ). This suggests that the maternal parents of these two fish were steelhead. In the remaining seven resident rainbow trout, Sr/Ca ratios did not differ significantly between the primordia and the freshwater growth regions ( $P > 0.05$ , range 0.09–0.4), indicating that the maternal parents were resident rainbow trout.

The distribution of Sr/Ca ratios in primordia sample points in steelhead and rainbow trout adults from the Deschutes River was bimodal and overlapping (Fig. 4). Similarly, the distribution of differences between mean Sr/Ca ratios in the primordia and mean Sr/Ca ratios in the freshwater growth region were bimodal in samples from both the Babine and Deschutes rivers (Fig. 5). In the Babine River sample, there was a slight overlap in differences, owing to the occurrence of resident rainbow trout of steelhead origin and steelhead of resident rainbow trout origin (Fig. 5a). There was no overlap in the Deschutes River samples (Fig. 5b).

**Fig. 5.** Frequency distribution of the difference between mean primordia Sr/Ca ratios and mean freshwater growth region Sr/Ca ratios for adult resident rainbow trout (solid bars) and adult steelhead (open bars) in the Babine River, British Columbia (a), and the Deschutes River, Oregon (b).



## Discussion

Given the temporal and spatial distribution (as measured by microhabitat characteristics) of spawning by steelhead and resident rainbow trout, we conclude that steelhead and resident rainbow trout segregate spawning habitat in the Deschutes River. There is a weak overlap in the timing of spawning but microhabitat selection appears to reinforce segregation. Based on maternal origin, as determined from otolith microchemistry, all adult steelhead from the Deschutes River were progeny of steelhead females and all resident rainbow trout were progeny of resident rainbow trout. This was not true for all individuals from the Babine River. The majority of individuals from the Babine River had the same life history as the maternal parent. However, one of the steelhead appeared to have had a resident maternal parent and two of the resident rainbow trout were the progeny of a steelhead maternal parent. These patterns suggest that there are differences between the Deschutes and Babine rivers concerning the degree of segregation between steelhead and resident rainbow trout.

In addition to temporal and spatial segregation of spawning, assortative mating has the potential to reinforce the limitation of gene flow between life-history forms of a species if the forms are different sizes. Foote and Larkin (1988)

determined that assortative mating served to reproductively isolate sockeye salmon and kokanee (*O. nerka*). During this study, only two instances of spawning activity between steelhead and resident rainbow trout were observed. Both instances occurred on the same day, 1 week after any other steelhead were observed spawning in the study sites. Steelhead adults captured in the Pelton Fish Trap (located at the Reregulating Dam) were released back into the river after sitting in the trap for at least 3 weeks. One day after being released in the river, they were observed spawning with rainbow trout. Generally, steelhead spawning occurred at night and resident rainbow trout spawning occurred during the day. "Sneaking" by small precocial males is likely to occur and would be difficult to detect. We cannot rule out the possibility of interbreeding between steelhead and resident rainbow trout through sneaking male parr.

Identification of maternal origin based on Sr/Ca ratios can be confounded by high Sr/Ca ratios in fresh waters. For example, Rieman et al. (1994) cautioned that the utility of otolith microchemistry is highly dependent on the variation in chemistry of fresh waters. Reiman et al. (1994) reported Sr/Ca ratios ranging from <0.0012 to 0.0061 for three lakes and a hatchery. The 0.0061 measurement from Alturas Lake precluded using Sr/Ca ratios to determine the maternal origin of sockeye salmon in that system. The Sr/Ca ratio in the freshwater growth regions and primordia of fish from Alturas Lake were similar to measurements in the primordia of known anadromous fish from other systems. As a result, Rieman et al. (1994) suggested that water chemistry be analyzed in future applications of otolith microchemistry. Sr/Ca ratios in the Deschutes River ranged from 0.0016 to 0.0030 in 13 quarterly samples collected between April 1983 and June 1986 (Alexander et al. 1996). Assuming that these samples represent the typical variation for the Deschutes River, Sr/Ca levels in the Deschutes River are low enough to allow otolith microchemistry to be used in determining maternal origin in steelhead and resident rainbow trout. Future research concerning the relationship of environmental and otolith Sr/Ca ratios in controlled conditions is warranted, to refine the utility of otolith microchemistry.

Although there is an overlap in the distribution of Sr/Ca ratios in individual sampling locations within the primordia of steelhead and resident rainbow trout, this does not preclude discrimination between the two life-history forms. Rather, it suggests that a single point sampled in a single primordium of a fish cannot discriminate life history. It is for this reason that we sampled as many primordia as possible within each fish for comparison with the freshwater growth region. In addition, comparison of Sr/Ca ratios in the primordia and freshwater growth regions can only identify maternal origin. Therefore, it is unknown whether male resident rainbow trout contributed to the anadromous population or whether male anadromous trout contributed to the resident population. It is possible that gene flow between life-history forms may occur in this manner.

We used the lack of steelhead of resident maternal origin and residents of steelhead maternal origin to confirm that the temporal and spatial segregation observed in spawning in the Deschutes River could potentially lead to reproductive isolation. If the progeny of steelhead were contributing to the adult resident population and vice versa, the segregation in



spawning could not in fact lead to reproductive isolation. In combination with the spatial and temporal segregation of spawning, it suggests that the life-history forms may constitute separate isolated populations. Observations from acquired markers such as otolith microchemistry cannot be unequivocally equated with reproductive isolation unless this pattern is confirmed to exist over many generations but, in combination with other segregating mechanisms, it can suggest the possibility of reproductive isolation.

Given the apparent reproductive isolation between steelhead and resident rainbow trout in the Deschutes River that results from segregation of spawning habitat, divergence between the two life-history forms should be evident in genetic differences. Chilcote (1976) compared steelhead and resident rainbow trout from five locations within the lower Deschutes River, Oregon, based on allele frequencies at 13 loci examined with protein electrophoresis. No significant genetic differences were observed, with the exception of one headwater population of resident trout isolated above two waterfalls. Chilcote (1976) concluded, therefore, that considerable interbreeding and gene flow occurred between Deschutes River steelhead and resident rainbow trout. Currens et al. (1990) re-examined the relationship of rainbow trout populations among locations within the lower Deschutes River based on allele frequencies at 24 loci examined with protein electrophoresis and meristic analysis. Currens et al. (1990) and Currens (1987) did find genetic distinction between resident rainbow trout and steelhead isolated by barrier falls and suggested that resident rainbow trout may derive from either resident or anadromous forms, although some isolation between these forms was detected. Although genetic differences have not yet been described between what appear to be reproductively isolated populations of steelhead and resident rainbow trout in the Deschutes River (similar to the situation described by Ryman et al. (1979)), such differences cannot be ruled out. Newer molecular-genetic methods should be applied to steelhead and resident rainbow trout.

It has been suggested that resident populations can give rise to anadromous juveniles (Northcote 1992). For example, Rieman et al. (1994) found juveniles of both kokanee and sockeye origin in samples of emigrants (presumed smolts) leaving Redfish Lake, Idaho. However, of the five adult sockeye salmon that returned to Redfish Lake in 1991, all had had a sockeye maternal parent. Since 1991, adult sockeye of kokanee maternal origin have been confirmed at Redfish Lake (Bruce Rieman, U.S. Forest Service, Rocky Mountain Research Station, Boise, Idaho, personal communication). In presumed steelhead smolts collected from the estuary of a small central California coastal stream, juveniles of both steelhead and resident rainbow trout maternal origin were present (C.E. Zimmerman, unpublished data). This observation suggests that resident rainbow trout may contribute to the recruitment of smolts, but only examination of adult steelhead can confirm that resident fish contribute to the recruitment of adult steelhead. The selection pressures on resident rainbow trout and steelhead are likely to be very different. Experimental studies with sockeye salmon and kokanee have demonstrated behavioral, ecological, and developmental differences between the two forms (Wood and Foote 1990). This is an important avenue of future research on the dynamics of sympatric steelhead and rainbow trout.

Reproductive isolation among sympatric life-history morphs of the same species has been described for several salmonids, including kokanee (Kurenkov 1978), brown trout (Baglinière et al. 1989; Skaala and Nævdal 1989), and Atlantic salmon (Verspoor and Cole 1989). The two forms of kokanee described by Kurenkov (1978) were reproductively isolated within a single lake. Spawning by the two populations was spatially and temporally segregated and the two populations exhibited trophic and morphologic differences. In locations where spawning by the two forms overlapped spatially, there was a temporal segregation of spawning. Baglinière et al. (1989) also described spatial segregation of spawning by anadromous and resident brown trout based on spawning locations and the distribution of tagged fish.

Reproductive isolation between life-history forms has also been described in other fish families. For example, Snyder and Dingle (1990) investigated threespine stickleback (*Gasterosteus aculeatus*) populations from the Navarro River, California. This species was divided into a freshwater resident population that migrated <5 km and spawned in small tributaries and an anadromous population that migrated between the estuary and spawned in lower reaches of the mainstem Navarro River. Rearing experiments indicated that life history (freshwater resident vs. anadromous) was genetically based. Taylor and Bentzen (1993) used molecular-genetic evidence to determine reproductive isolation between sympatric "normal-sized" and "dwarf-sized" rainbow smelt (*Osmerus mordax*) in a New Brunswick lake. Taylor and Bentzen (1993) argued that, given the molecular evidence of reproductive isolation coupled with strong morphological and ecological differences, the two forms of smelt were behaving as distinct species. Bernatchez et al. (1996) described similar reproductive isolation among whitefish (*Coregonus clupeaformis*) trophic ecotypes in Yukon lakes. Bernatchez et al. (1996) suggested that the molecular data corroborated previous genetic and ecological studies that demonstrated reproductive isolation between sympatric trophic ecotypes of whitefish.

Reproductive isolation may lead to genetic divergence among sympatric populations under different selection regimes. The selective pressure experienced by anadromous and nonanadromous individuals are probably very different and would be expected to lead to genetic differences between the two forms (Foote et al. 1989). Very few studies have examined the relationship of sympatric steelhead and resident rainbow trout and the results of these studies vary. Neave (1944) examined steelhead and rainbow trout from the Cowichan River in British Columbia and identified significant differences in meristic characters. These differences were maintained in experimental conditions, leading Neave to conclude that the differences were hereditary and that the two life-history forms (steelhead and resident rainbow trout) should be treated as two different species. On the other hand, Savvaitova et al. (1997) concluded that the three life-history forms (resident, estuary migrating, and anadromous) of rainbow trout present in the Utkholok River of western Kamchatka represented one interbreeding population. Given the disparity in results among locations, it is likely that the population structure is not species-specific but, rather, dictated by a combination of environment conditions, phylogeny, and genetics.

Many studies examining reproductive isolation among life-

history forms of the same species have inferred reproductive isolation based solely on genetic analyses or conjecture. Verspoor and Cole (1989), for example, speculated that temporal and spatial segregation of spawning led to genetic differentiation between resident and anadromous populations of Atlantic salmon in Newfoundland, Canada. On the other hand, Ryman et al. (1979) found little detectable genetic differentiation between brown trout populations that were assumed to be reproductively isolated. Although reproductive isolation may have led to or maintained the differences observed by Verspoor and Cole (1989), the occurrence of isolation should be independently corroborated. Indirect measures of reproductive isolation among life-history forms should not be considered the final arbiter. Spatial and temporal segregation or assortative mating should also be used to identify the presence and degree of reproductive isolation.

Whether sympatric life-history forms are managed as single populations exhibiting polymorphism or as reproductively isolated populations has profound implications in decisions related to protection and recovery of species. For example, in considering the status of coastal cutthroat trout in the North Umpqua River in Oregon, the resident and sea-run forms were treated as one single unit (Waples 1995), under the assumption that each life-history form could give rise to the other. This would not be appropriate for Deschutes River steelhead and resident rainbow trout, which, given the segregation of spawning habitat and potential reproductive isolation, should be treated as two reproductively isolated populations. As a result, there is little chance that resident rainbow trout can contribute to the recovery of steelhead in the Deschutes River. In locations where steelhead and resident rainbow trout are not reproductively isolated, the recovery of one life-history form of the population from the other life-history form may be a possible conservation strategy. Although these results do not unequivocally describe the relationship of steelhead and resident rainbow trout, they do suggest that there may be variation among locations in this relationship.

## Acknowledgements

Funding was provided by Portland General Electric Corporation and the U.S. Forest Service Pacific Northwest Research Station, Corvallis, Oregon. The Confederated Tribes of the Warm Springs Indian Reservation kindly allowed access to tribal lands. Don Ratliff was instrumental in the formation of this study, and discussions with Ken Currens improved this study. Numerous people assisted with field work, including Michelle St. Peters, Rich Madden, Eric Schulz, Jim Eisner, and Colleen Fagan. We thank Bob Hooton of the British Columbia Ministry of Environment, Land, and Parks for providing otoliths of steelhead and rainbow trout from the Babine River. Jim Newton assisted with collection of adult rainbow trout. We appreciate the comments of Doug Markle, Mark Hixon, Roger Nielsen, Hiram Li, Jim Hall, Bruce Rieman, and three anonymous reviewers that improved the manuscript. Roger Nielsen provided instruction and assistance with microprobe techniques. This work is a portion of a Doctoral dissertation by C.E.Z. in the Department of Fisheries and Wildlife at Oregon State University.

## References

- Alexander, R.B., Slack, J.R., Ludtke, A.S., Fitzgerald, K.K., and Schertz, T.L. 1996. Data from selected U.S. Geological Survey national stream water-quality monitoring networks (WQN) on CD-ROM. U.S. Geol. Surv. Open-File Rep. No. 96-337.
- Babaluk, J.A., Halden, N.M., Reist, J.D., Kristofferson, A.H., Campbell, J.L., and Teesdale, W.J. 1997. Evidence for non-anadromous behaviour of Arctic charr (*Salvelinus alpinus*) from Lake Hazen, Ellesmere Island, Northwest Territories, Canada, based on scanning proton microprobe analysis of otolith strontium distribution. *Arctic*, **50**: 224–233.
- Baglinière, J.L., Maisse, G., Lebail, P.Y., and Nihouarn, A. 1989. Population dynamics of brown trout, *Salmo trutta* L., in a tributary in Brittany (France): spawning and juveniles. *J. Fish Biol.* **34**: 97–110.
- Behnke, R.J. 1992. Native trout of western North America. *Am. Fish. Soc. Monogr.* No. 6.
- Bernatchez, L., Vuorinen, J.A., Bodaly, R.A., and Dodson, J.J. 1996. Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (*Coregonus*). *Evolution*, **50**: 624–635.
- Berthold, P. 1991. Genetic control of migratory behaviour in birds. *Trends Ecol. Evol.* **6**: 254–257.
- Chilcote, M.W. 1976. Genetic comparison of Deschutes River steelhead and rainbow trout at selected enzyme loci. M.S. thesis, Oregon State University, Corvallis.
- Currens, K.P. 1987. Genetic differentiation of resident and anadromous rainbow trout (*Salmo gairdneri*) in the Deschutes River, Oregon. M.S. thesis, Oregon State University, Corvallis.
- Currens, K.P., Schreck, C.B., and Li, H.W. 1990. Allozyme and morphological divergence of rainbow trout (*Oncorhynchus mykiss*) above and below waterfalls in the Deschutes River, Oregon. *Copeia*, 1990: 730–746.
- Foot, C.J., and Larkin, P.A. 1988. The role of mate choice in the assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). *Behaviour*, **106**: 43–62.
- Foot, C.J., Wood, C.C., and Withler, R.E. 1989. Biochemical genetic comparison of sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka*. *Can. J. Fish. Aquat. Sci.* **46**: 149–158.
- Gross, M.R. 1987. Evolution of diadromy in fishes. *Am. Fish. Soc. Symp.* **1**: 14–25.
- Henshaw, F.F., Lewis, J.H., and McCaustland, E.J. 1914. Deschutes River, Oregon, and its utilization. U.S. Geol. Surv. Water-Supply Pap. No. 344.
- Huntington, C.W. 1985. Deschutes River spawning gravel study: final report. Bonneville Power Administration, Division of Fish and Wildlife, Project No. 83-423. Bonneville Power Administration, P.O. Box 3261, Portland, OR 97208.
- Jonsson, B., and Jonsson, N. 1993. Partial migration: niche shift versus sexual maturation in fishes. *Rev. Fish Biol. Fish.* **3**: 348–365.
- Jordan, D.S., and Evermann, B.W. 1905. American food and game fishes. Doubleday, Page, and Company, New York.
- Kalish, J.M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fish. Bull.* **88**: 657–666.
- Kurenkov, S.I. 1978. Two reproductively isolated groups of kokanee salmon, *Oncorhynchus nerka kennerlyi*, from Lake Kronotskiy. *J. Ichthyol. (Engl. Transl. [Vopr. Ikhtiol.])*, **17**: 526–534.
- Leider, S.A., Chilcote, M.W., and Loch, J.J. 1984. Spawning characteristics of sympatric populations of steelhead trout (*Salmo gairdneri*): evidence for partial reproductive isolation. *Can. J. Fish. Aquat. Sci.* **41**: 1454–1462.

- McKern, J.L., Horton, H.F., and Koski, K.V. 1974. Development of steelhead trout (*Salmo gairdneri*) otoliths and their use for age analysis and for separating summer from winter races and wild from hatchery stocks. *J. Fish. Res. Board Can.* **31**: 1420–1426.
- Narver, D.W. 1969. Age and size of steelhead trout in the Babine River, British Columbia. *J. Fish. Res. Board Can.* **26**: 2754–2760.
- Neave, F. 1944. Racial characteristics and migratory habits in *Salmo gairdneri*. *J. Fish. Res. Board Can.* **6**: 245–251.
- Nordeng, H. 1983. Solution to the “charr” problem based on Arctic charr (*Salvelinus alpinus*) in Norway. *Can. J. Fish. Aquat. Sci.* **40**: 1372–1387.
- Northcote, T.G. 1992. Migration and residency in stream salmonids—some ecological considerations and evolutionary consequences. *Nordic J. Freshw. Res.* **67**: 5–17.
- Olsen, E.A., Beamesderfer, P.M.P., McLean, M.L., and Tinus, E.S. 1994. Salmon and steelhead stock summaries for the Deschutes River Basin: an interim report. Oregon Department of Fish and Wildlife, Portland.
- Radtke, R.L. 1995. Otolith microchemistry of charr—use in life history studies. *Nordic J. Freshw. Res.* **71**: 392–395.
- Rieman, B.E., Myers, D.L., and Nielsen, R.L. 1994. Use of otolith microchemistry to discriminate *Oncorhynchus nerka* of resident and anadromous origin. *Can. J. Fish. Aquat. Sci.* **51**: 68–77.
- Ryman, N., Allendorf, F.W., and Ståhl, G. 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). *Genetics*, **92**: 247–262.
- Savvaitova, K.A., Kuzishchin, K.V., Maksimov, S.V., and Pavlov, S.D. 1997. Population structure of mikizha, *Salmo mykiss* in the Utkholok River (Western Kamchatka). *J. Ichthyol. (Engl. Transl. [Vopr. Ikhtiol.])*, **37**: 216–225.
- Schroeder, R.K., and Smith, L.H. 1989. Life history of rainbow trout and effects of angling regulations, Deschutes River, Oregon. Inf. Rep. No. 89-6 of the Oregon Department of Fish and Wildlife, Portland.
- Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. *Fish. Res. Board Can. Bull. No.* 184.
- Secor, D.H., Henderson-Arzapalo, A., and Piccoli, P.M. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *J. Exp. Mar. Biol. Ecol.* No. 192, pp. 15–33.
- Skaala, Ø., and Nævdal, G. 1989. Genetic differentiation between freshwater resident and anadromous brown trout *Salmo trutta*, within watercourses. *J. Fish Biol.* **34**: 597–605.
- Snyder, R.J., and Dingle, H. 1990. Effects of freshwater and marine overwintering environments on life histories of threespine sticklebacks: evidence for adaptive variation between anadromous and resident populations. *Oecologia*, **84**: 386–390.
- Taylor, E.B., and Bentzen, P. 1993. Molecular genetic evidence for reproductive isolation between sympatric populations of smelt (*Osmerus*) in Lake Utopia, south-western New Brunswick, Canada. *Mol. Ecol.* **2**: 345–357.
- Thorpe, J.E. 1987. Smolting versus residency: developmental conflicts in salmonids. *Am. Fish. Soc. Symp.* **1**: 244–252.
- Toole, C.L., and Nielsen, R.L. 1992. Effects of microprobe precision on hypotheses related to otolith Sr:Ca ratios. *Fish. Bull.* **90**: 421–427.
- Verspoor, E., and Cole, L.J. 1989. Genetically distinct sympatric populations of resident and anadromous Atlantic salmon, *Salmo salar*. *Can. J. Zool.* **67**: 1453–1461.
- Waples, R.S. 1995. Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. *In* Evolution and the aquatic ecosystem: defining unique units in population conservation. *Edited by* J.L. Nielsen. *Am. Fish. Soc. Symp.* **17**: 8–27.
- Wood, C.C. 1995. Life history variation and population structure in sockeye salmon. *In* Evolution and the aquatic ecosystem: defining unique units in population conservation. *Edited by* J.L. Nielsen. *Am. Fish. Soc. Symp.* **17**: 195–216.
- Wood, C.C., and Foote, C.J. 1990. Genetic differences in the early development and growth of sympatric sockeye salmon and kokanee (*Oncorhynchus nerka*). *Can. J. Fish. Aquat. Sci.* **47**: 2250–2260.
- Zimmerman, C.E., Currens, K.P., and Reeves, G.H. 1997. Genetic population structure of coastal cutthroat trout in the Muck Creek basin, Washington. *In* Sea-run cutthroat trout: biology, management and future conservation. *Edited by* J.D. Hall, P.A. Bisson, and R.E. Gresswell. Oregon Chapter, American Fisheries Society, Corvallis, Oregon. pp. 170–172.